



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : A61K 38/29	A2	(11) International Publication Number: WO 99/12561 (43) International Publication Date: 18 March 1999 (18.03.99)
(21) International Application Number: PCT/EP98/05550 (22) International Filing Date: 2 September 1998 (02.09.98) (30) Priority Data: 60/058,324 9 September 1997 (09.09.97) US (71) Applicant: F. HOFFMAN-LA ROCHE AG [CH/CH]; 124 Grenzacherstrasse, CH-4070 Basel (CH). (72) Inventor: VICKERY, Brian; Henry; 27977 Via Ventana, Los Altos Hills, CA 94022 (US). (74) Agent: BRAUN, Axel; 124 Grenzacherstrasse, CH-4070 Basel (CH).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>Without international search report and to be republished upon receipt of that report.</i> BEST AVAILABLE COPY
(54) Title: FRACTURE HEALING USING PTHrP ANALOGS (57) Abstract The present invention provides methods of bone healing and fracture repair comprising administering to a patient in need thereof an effective amount of a polypeptide analog of parathyroid hormone related peptide (PTHrP) and salts thereof, wherein amino acid residues 22-31 form an amphipathic α -helix or the use of such a polypeptide for the preparation of a medicament for the treatment of bone healing and fracture repair.		

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

Fracture healing using Pthrp Analogs

Approximately 8-10 million bone fractures are reported annually in the United States with more than 1 million of these requiring hospitalization. The estimated annual costs of treating these fractures exceeds 20 billion dollars. While this is already significant, these numbers are expected to increase due to the aging of the general population. Even though several therapies are indicated for preventing the bone loss associated with aging, there are fewer therapies indicated for treatment once a fracture has occurred. Most of these require local administration which is undesirable due to the complexity of delivery and poor patient compliance. Therefore, it would be desirable to have additional methods of facilitating bone healing and fracture repair.

It has recently been reported that intermittent treatment with parathyroid hormone (PTH) improves fracture healing in ovariectomized rats, indicating that PTH treatment may be potentially useful in treating postmenopausal osteoporotic fractures. (H.W. Kim *et al.*; Transactions of the 43rd Annual Meeting of the Orthopaedic Research Society, Vol. 22, Section 1, Abstract 181-31, February 9-13, 1997; H.W. Kim *et al.*; Journal of Bone and Mineral Research, Vol. 11, Supplement 1, page S152, Abstract P248 (August 1996)). Other investigators have reported that implantation of a gene activated matrix expressing bone morphogenetic protein-4 and/or a fragment of PTH (amino acids 1-34) into the segmented defect rat fracture model causes formation of new bone which bridges the gap more rapidly than an untreated control. (Jianming Fang *et al.*; Proc. Natl. Acad. Sci. (USA), Vol. 93:5753-5758 (June 1996)). Various PTH analogs have also been reported to be useful for treatment of osteoporosis (U.S. Patent Nos. 5,556,940 and 5,559,792). Other methods of fracture healing include the use of human platelet factor 4 (U.S. Patent No. 5,622,935), benzothienophenes (U.S. Patent No. 5,502,074) and 24,25(OH)₂ vitamin D₃ (U.S. Patent No. 5,069,905).

PTH related peptide (PTHrP), previously known as the factor responsible for humoral hypercalcemia of malignancy, is a peptide of 138-174 amino acids (depending on alternative splicing) which binds to the PTH/PTHrP receptor. The N-terminal 34 amino acid sequence of PTHrP is of limited sequence

homology to that of PTH, but in certain cases shows similar activity to PTH. However, PTHrP is generally less potent and less bone anabolic than PTH and has not been associated with fracture healing. The sequence of hPTHrP (1-34) is as follows:

5 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile
 1 5 10 15
 Gln Asp Leu Arg Arg Arg Phe Phe Leu His His Leu Ile Ala Glu
 20 25 30
 Ile His Thr Ala (SEQ ID NO:1).

10 Several truncated homologs and analogs of PTHrP have been reported. Analogs in which amino acid residues 22-31 of PTHrP(1-34) are replaced by an amphipathic α -helix (U.S. Patent No. 5,589,452 and WO 97/07815) and related derivatives have been described as useful for treating osteoporosis. (B. H. Vickery *et al.* J.Bone & Mineral Research, 11(12):1943-1951 (1996); D. Leaffer
 15 *et al.* Endocrinology, 136(8):3624-3631 (1995)). Monocyclic and bicyclic analogs of PTHrP (1-34) and PTHrP(7-34) were shown to bind strongly to the PTH receptor and stimulate (or antagonise) PTH-stimulated adenyl cyclase activity in osteoblast-like cells. (Michael Chorev *et al.* Biochemistry, 36:3293-3299 (1997), and WO 96/40193).

20 In one aspect, this invention provides methods of bone healing and fracture repair comprising administering to a patient in need thereof an effective amount of a polypeptide analog of parathyroid hormone related peptide (PTHrP) and salts thereof, wherein amino acid residues 22-31 form an amphipathic α -helix, preferably composed of hydrophilic amino acids (Haa)
 25 and lipophilic amino acids (Laa) ordered in the sequence:

Haa (Laa Laa Haa Laa)₂ Laa;

or the use of such compounds for the preparation of a medicament for the treatment of such disorders.

30 When illustrative embodiments of this amphipathic helix are inserted into the PTHrP sequence, particularly into N-terminal truncates of human PTHrP (residues 1-32 through 1-38), the resulting polypeptides are effective in bone healing and fracture repair. Systemic administration is a preferred mode of delivery.

A brief description of the drawings is given below:

Figure 1 shows the weekly radiographic density of a segmented bone defect in the rat fracture model over the course of five weeks of treatment with PTHrP analogs B or C relative to control. It shows that after four weeks, bone growth in the defects of animals treated with the PTHrP analogs was greater than in the control animals. PTHrP analogs B and C are $(\text{MAP}_{1-10})^{22-31}\text{Ala}^{19}\text{hPTHrP}(1-34)\text{NH}_2$ and $(\text{MAP}_{1-10})^{22-31}\text{His}^{26}\text{hPTHrP}(1-34)\text{NH}_2$ respectively.

Figure 2 shows the radiographic density ratio of the segmented bone to the contralateral femur (control) in the segmented defect rat fracture model after six weeks of treatment with control or PTHrP analogs B or C. Rats treated with PTHrP analogs B and C had an increased bone density relative to control treated rats.

Figure 3 shows high resolution radiographs of bone defects in the segmented defect rat fracture model of rats after treatment for six weeks with either control (3A) or PTHrP analog C (3B). The radiograph demonstrates that the control defect remains non-united whereas the defect treated with PTHrP analog C has healed.

Figures 4A and 4B show the healing of an intramembraneous bone defect in rabbits after treatment with PTHrP analog D, $(\text{MAP}_{1-10})^{22-31}\text{hPTHrP}(1-34)\text{NH}_2$ at two different doses.

Figure 5 shows the union rate achieved in the corticosteroid induced delayed healing fracture model with PTHrP analog D, $(\text{MAP}_{1-10})^{22-31}\text{hPTHrP}(1-34)\text{NH}_2$.

In the following a more detailed description of the invention is given.

The one- and three-letter abbreviations for the various common nucleotide bases and amino acids are as recommended in Pure Appl. Chem. 31: 639-645 (1972) and 40: 277-290 (1974). The abbreviations represent L amino acids unless otherwise designated as D or D,L. Certain amino acids, both natural and non natural, are achiral, e.g. glycine. All peptide sequences are presented with the N terminal amino acid on the left and the C terminal amino acid on the right.

It will be recognized that both natural and unnatural amino acids may be present in the PTHrP analogs used in this invention. Examples of unnatural amino acids and their abbreviations include, homoserine (hSer), homoserine lactone (hSerlac), homocysteine (Hcy), homoarginine (hArg), homocitrulline (Hci), penicillamine (Pen), N α -methylarginine (N-MeArg), norleucine (Nle),

norvaline (Nval), norisoleucine (Nile), N-methylisoleucine (N-MeIle), phenylglycine (PhG), t-butylglycine (Tle), hydroxyproline (Hyp), 3,4-dehydropipecolinic acid (Δ -Pro), pyroglutamine (Pyr, Glp), ornithine (Orn), , 1-aminoisobutyric acid (1-Aib), 2-aminoisobutyric acid (2-Aib), 2-aminobutyric acid (2-Abu), 4-aminobutyric acid (4-Abu), 2,4-diaminobutyric acid (A2bu), α -aminosuberic acid (Asu), albizzin (Abz), β -cyclohexylalanine (Cha), 3-(1-naphthyl)alanine (1-Nal), 3-(2-naphthyl)alanine (2-Nal), citrulline (Cit), pipercolinic acid (Pip), 4-chlorophenylalanine (4-ClPhe), 4-fluorophenylalanine (4-FPhe), sarcosine (Sar) and 1-aminopropanecarboxylic acid (1-NCPC). Both natural and unnatural amino acids are commercially available from vendors such as NovaBiochem (San Diego, CA, USA) and Bachem (Torrance, CA, USA).

The PTHrP polypeptide analogs are described with reference to their variation from the native sequence of hPTHrP. The representation (MAP₁₋₁₀) refers to the particular amphipathic helical sequence shown below.

Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu (SEQ ID NO:2)

the MAP sequence of ten amino acid residues.

Thus, the sequence represented as (MAP₁₋₁₀)²²⁻³¹hPTHrP(1-34) refers to the 1-34 N-terminal residues of hPTHrP with the segment between residues 22-31 replaced by the MAP sequence. Within the MAP sequence, additional variants may be denoted. Thus, (MAP₁₋₁₀)²²⁻³¹His²⁶hPTHrP(1-34) refers to the 1-34 N-terminal residues of hPTHrP with the segment between residues 22-31 replaced by a MAP sequence in which there is a histidine at position 26 (instead of lysine as in the standard MAP sequence).

Additional variants from the naturally occurring sequence are similarly denoted. (MAP₁₋₁₀)²²⁻³¹Pro³²hPTHrP(1-32) refers to the 1-32 N-terminal residues of hPTHrP with the MAP sequence at positions 22-31 and a proline (replacing the naturally occurring histidine) at position 32. (MAP₁₋₁₀)²²⁻³¹D-Orn³⁴lactam hPTHrP (1-34) refers to the 1-34 N-terminal residues of hPTHrP with the MAP sequence at positions 22-31 and an ornithine at position 34 with a lactam formed between the amino group of the ornithine side chain and the carboxy terminus. (MAP₁₋₁₀)²²⁻³¹hSer³⁴hPTHrP(1-34)Thr His Ile Gln NH₂ refers to the 1-34 N-terminal residues of hPTHrP with the MAP sequence at positions 22-31, a homoserine at position 34, an additional sequence Thr His Ile Gln at positions 35-38 and the carboxy terminus being a primary amide.

Polypeptides with cyclized links between amino acid residues are denoted with the two linked residues placed within brackets and preceded by a "c." In general, the links between the two residues are formed between the side chain functionalities, typically as amide or ester bonds. Thus (MAP₁₋₁₀)²²⁻
5 ³¹c[Lys¹³,Asp¹⁷]hPTHrP(1-34) hSer³⁴ lactone refers to the 1-34 N-terminal residues of hPTHrP with the segment between residues 22-31 replaced by the MAP sequence, the amino side chain of lysine at position 13 cyclized with the carboxyl side chain of aspartate at position 17 and an additional homoserine at position 34 with a lactone formed between the hydroxyl side chain of the
10 homoserine and the carboxyl terminus. Similarly, (MAP₁₋₁₀)²²⁻³¹ c[Lys¹³,Asp¹⁷]hPTHrP(1-34) refers to the 1-34 N-terminal residues of hPTHrP with the segment between residues 22-31 replaced by the MAP sequence and the amino side chain of a lysine at position 13 cyclized with the carboxyl side chain of an aspartate at position 17.

15 "Hydrophilic amino acid (Haa)" refers to an amino acid having at least one hydrophilic functional group in addition to those required for peptide bond formation, such as arginine, asparagine, aspartic acid, glutamic acid, glutamine, histidine, lysine, serine, threonine, and their homologs.

"Lipophilic amino acid (Laa)" refers to an uncharged, aliphatic or
20 aromatic amino acid, such as isoleucine, leucine, methionine, phenylalanine, tryptophan, tyrosine, valine, and their homologs.

For the purposes of this invention, alanine is classified as "amphiphilic" i.e., capable of acting as either hydrophilic or lipophilic.

A "polypeptide analog of PTHrP" refers to a polypeptide having art-
25 accepted substitutions, deletions or insertions relative to PTHrP or is substantially homologous to PTHrP such that the analog has a similar physiological activity.

"Physiologically active truncated analog of PTHrP" refers to a polypeptide having a sequence comprising less than the full complement of
30 amino acids found in PTHrP which, however, elicits a similar physiological response. The truncated PTHrP analogs need not be fully homologous with PTHrP to elicit a similar physiological response. Typically, the truncated analogs will be truncated from the C-terminus and will range from 30 to 40 residues, with PTHrP(1-32), PTHrP(1-34) and PTHrP(1-38) being preferred,
35 but not exclusive, representatives of this group. Generally, the analogs will

carry conservative substitutions of amino acids according to art-accepted parameters as described below.

The term "substantially homologous," when referring to polypeptides, indicates that the polypeptide in question exhibits at least about 80%
5 homology, usually about 90% homology, and preferably 95% homology to the referenced polypeptide. Homology for polypeptides is typically measured using sequence analysis software. (See, e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wisconsin 53705 USA).

10 "Amphipathic α -helix" refers to the secondary structure exhibited by certain polypeptides in which the amino acids assume an α -helical configuration having opposing polar and nonpolar faces oriented along the long axis of the helix. Amphipathic helical sequences can be designed by those of skill in the art. Particular amphipathic helical sequences suitable for use in
15 the methods of this invention are described in more detail in, e.g. U.S. Patent No.5,589,452 or WO 97/07815.

The present invention is based upon the finding that certain analogs of PTHrP containing an amphipathic α -helix at positions 22-31 are effective in bone healing and fracture repair. The amphipathic α -helix is composed of
20 hydrophilic amino acids (Haa) and lipophilic amino acids (Laa) ordered in the sequence:

Haa (Laa Laa Haa Laa)₂ Laa

25 When illustrative embodiments of this amphipathic helix are inserted into the PTHrP sequence, particularly into N-terminal truncates of human PTHrP (residues 1-32 through 1-38), the resulting polypeptides are effective in bone healing and fracture repair. Unlike PTH, PTHrP or analogs thereof not having this amphipathic helical segment, the analogs used herein do not cause
30 hypercalcemia. In addition, these analogs induce a more rapid increase in bone relative to either PTH or PTHrP.

It will be recognized that in addition to containing an amphipathic helix between positions 22 and 31, a variety of substitutions, deletions and insertions of amino acids may be made in the PTHrP sequence outside this
35 region while still preserving the three dimensional structure of the

polypeptide. Representative variations of the PTH and PTHrP sequence which maintain the physiological activity of the resulting analogs are disclosed in U.S. Patent Nos. 5,599,792, 5,556,940, 5,607,915 and 5,589,452, and WO 91/06564, WO 94/02510, WO 95/11697, WO 96/40193 and WO 5 97/07815. Additional variants which will be expected by one of skill in the art to maintain physiological activity can be made by following art-accepted protein structure modeling techniques. Representative methodologies for deriving such variants are described *inter alia* by I. Ladunga and R.F. Smith, Protein Eng., 3:187-196 (1997) and by M.J. Thompson and R.A. Goldstein, in 10 Proteins, 1:28-37 (1996).

Substitutional variants are those in which at least one amino acid in the native sequence is removed and a different amino acid is put into its place at the same position. The substitutions may be single, where only one amino acid is replaced, or multiple where two or more amino acids are replaced in the 15 same molecule. It is generally expected that any conservative substitutions will be permitted. Thus, an analog corresponding to substituting one hydrophilic amino acid for another hydrophilic amino acid or a hydrophobic amino acid for another hydrophobic amino acid is expected to maintain similar fracture healing properties to its precursor. Substitutions also include PTHrP 20 analogs wherein the C-terminal residue is present as an amide. Additional substitutions may be made based on amino acids being either charged or uncharged. Each of these groups may be further divided into subgroups to further facilitate substitutions.

Charged amino acids

25 Acidic residues: aspartic acid, glutamic acid, 2-amino suberic acid

 Basic residues: lysine, arginine, histidine, ornithine

Uncharged amino acids

 Hydrophilic residues: serine, threonine, asparagine, glutamine, methionine

30 Aliphatic residues: glycine, alanine, valine, leucine, norleucine, isoleucine

 Nonpolar residues: cysteine, homocysteine, methionine, proline

 Aromatic residues: phenylalanine, tyrosine, tryptophan, histidine

 Alternatively, amino acid substitutions may be based on the principal of bioisosterism. Such bioisosteric substitutions typically minimize any

disruptive conformational effects that random substitution may create. The technique of alanine scanning may be used to identify positions where isosteric substitution is expected to provide variants which retain physiological activity. (See, e.g. K.H. Pearce Jr., M.H. Ultsch, R.F. Kelley,

- 5 A.M. de Vos and J.A. Wells, *Biochemistry*, **35** (32):10300-10307 (1996) and B. Li, J.Y. Tom, D Oare, R. Yen, W.J. Fairbrother, J.A. Wells and B.C. Cunningham, *Science*, **270**:1657-1660 (1995)). Representative isosteric amino acids are shown in the table below:

Amino acid	Isosteric amino acid
Ala	Ser, Gly
Glu	Gln, Asp
Gln	Asn, Glu
Asp	Asn, Glu
Asn	Ala, Asp
Leu	Met, Ile
Gly	Pro, Ala
Lys	Met, Arg
Ser	Thr, Ala
Val	Ile, Thr
Arg	Lys, Met, Asn
Thr	Ser, Val
Pro	Gly
Ile	Met, Leu, Val
Met	Ile, Leu
Phe	Tyr
Tyr	Phe
Cys	Ser, Ala
Trp	Phe,
His	Asn, Gln

Deletional variants are those with one or more amino acids in the native amino acid sequence removed. Ordinarily, deletional variants will have one or two amino acids deleted in a particular region of the molecule. It is likely that deletions will be made at the ends of the sequence, particularly the carboxy terminus. Thus, though PTHrP(1-34) fragments are preferred, sequences which are further truncated at the carboxyl terminus also have bone healing effects.

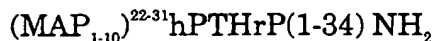
Insertional or addition variants are those with the amino acid inserted immediately adjacent to an amino acid at a particular position in the native sequence. Immediately adjacent to an amino acid means connected to either the α -carboxy or α -amino group of the amino acid. Addition or insertion variants are also likely to be made at the ends of the sequence, again most likely at the carboxy terminus.

Of the above-listed modifications to the native sequence, substitutions, and carboxy terminus additions and deletions are preferred.

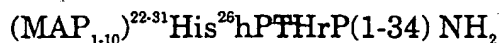
The polypeptide PTHrP analogs that are useful for fracture healing as described herein are generally described, in part, in U.S. Patent No. 5,589,452 and WO 97/07815. Additional analogs include the cyclic analogs of N-terminal hPTHrP(1-32), hPTHrP(1-34) and hPTHrP(1-38) containing a MAP sequence between positions 22 and 31 and optionally having residues at positions 13 and 17 and/or 26 and 30 linked via their side chain functionalities. It will be recognized by one of skill in the art that a variety of substitutions can be made at positions 13 and 17 which would allow cyclization between those two positions.

PTHrP analogs of any mammalian species, e.g., human, bovine, porcine or rabbit may be used in this invention, with human PTHrP being preferred. One of skill in the art will recognize that substitution, deletion and insertion variants of the preferred embodiments enumerated below, according to the art-accepted principles described above, are also within the scope of the invention.

Preferred embodiments include the use of hPTHrP(1-34) analogs with a MAP sequence at positions 22-31, particularly those having a positively charged amino acid at position 26, e.g., lysine or histidine. Specific embodiments within this class are:

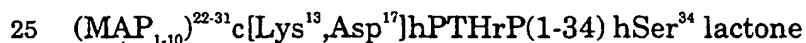


Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser IleGln Asp Leu Arg
 5 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
 NO:3) and



10 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser IleGln Asp Leu Arg
 Arg Arg Glu Leu Leu Glu His Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
 NO:4).

Other preferred embodiments include the use of hPTHrP(1-34) analogs
 15 with a MAP sequence at positions 22-31 and additionally containing a mono or
 bicyclic substructure created by cyclization between the side chain
 functionalities of the amino acid residues, particularly between residues 13
 and 17 or between residues 26 and 30. The side chain functionalities are
 typically amino, hydroxy or carboxyl groups and cyclization occurs via the
 20 formation of an amide or ester bond. Residues with amino functionality on the
 side chain include lysine and ornithine. Residues with carboxyl functionality
 on the side chain include aspartic acid and glutamic acid. Specific
 embodiments within this class include:



Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr hSerlac (SEQ ID
 NO:5) and

(MAP₁₋₁₀)²²⁻³¹c[Lys¹³,Asp¹⁷]hPTHrP(1-34) NH₂.

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
5 NO:6).

Other hPTHrP analogs useful in this invention are the N-terminal
sequences of between 30 to 50 residues, preferably from 1-32, 1-33, 1-34, 1-35,
1-36, 1-37 and 1-38, having the MAP sequence at residues 22-31 and
10 optionally having a one or more substitutions at position 5, 13, 17, 19, 26, 30,
32 or 34. Specific embodiments within this class include:

(MAP₁₋₁₀)²²⁻³¹Ile⁵hPTHrP(1-34) NH₂

Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
15 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
NO:7),

(MAP₁₋₁₀)²²⁻³¹Ala¹⁹hPTHrP(1-34) NH₂

Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Ala
20 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
NO:8),

(MAP₁₋₁₀)²²⁻³¹Pro³²hPTHrP(1-32) NH₂

Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Ala
25 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu Pro NH₂ (SEQ ID NO:9),

(MAP₁₋₁₀)²²⁻³¹D-Orn³⁴lactam hPTHrP (1-34)

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr D-Orn
30 lactam(SEQ ID NO:10),

(MAP₁₋₁₀)²²⁻³¹hPTHrP (1-34) A2bu³⁴lactam

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr A2bu lactam(SEQ
35 ID NO:11) and

(MAP₁₋₁₀)²²⁻³¹hSer³⁴PTHrP(1-34)THIQ NH₂

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser IleGln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr hSer Thr His Ile
5 Gln NH₂ (SEQ ID NO 12).

Generally, all polypeptides substantially homologous to the specific
embodiments disclosed herein are useful for the methods of the invention.
Ordinarily, the polypeptides used in the present invention will be at least
10 about 50%, preferably in excess of about 80%, and, more preferably in excess
of about 90%, most preferably at least about 95% homologous to the specific
embodiments disclosed herein. The length of polypeptide sequences compared
for homology will be generally at least about 20 amino acids, usually at least
about 24 residues, typically at least about 30 residues and preferably between
15 32 and 40 residues.

The compounds used in the present invention can be made by methods
described in U.S. Patent No. 5,589,452, and WO 96/40193 and WO 97/07815.
The compounds are generally made by solid phase synthesis, solution
synthesis or recombinant methods which proceed via the cloning and
20 expression of a gene coding for the polypeptide of interest, all known to one of
skill in the art. Solid phase syntheses are preferred for truncated PTHrP
analogs of forty or fewer amino acids. Analogs with cyclized side chains are
generally prepared by assembling the complete protected polypeptide on the
resin, removing the protecting groups and effecting the cyclization with an
25 appropriate coupling agent, such as benzotriazol-1-yl-
oxytris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP) just before
releasing the polypeptide from the resin.

The methods of treatment disclosed herein may be used for healing of
bone fractures and osteotomies, including both union and nonunion fractures.
30 Types of fractures treatable by the methods of this invention include both
traumatic and osteoporotic fractures, e.g., fractures of the hip, neck of the
femur, wrist, vertebrae, spine, ribs, sternum, larynx and trachea, radius/ulna,
tibia, patella, clavicle, pelvis, humerus, lower leg, fingers and toes, face and
ankle. Other uses include facilitating joint fusions, e.g., fusions of the spine,
35 ankle and foot, elbow, hip and arthrodeses of the hip, knee and shoulder.
Treatment with the PTHrP analogs as described herein is also indicated in

conjunction with arthroplastic procedures (including revision arthroplasties) of the hip, knee, shoulder/elbow etc. Bone healing may also be enhanced in other surgical settings such as in cranio and maxillofacial surgery, dental surgery and bunionectomy. PTHrP analogs have been shown to accelerate healing
5 both in endochondral bone (Examples 1 and 2) which proceeds via cartilagenous callous formation, as well as in intramembraneous bone (Example 3) which does not require intermediate callous formation. Healing in intramembraneous bone is particularly useful in cases where fracture healing is delayed, e.g., in diabetics, smokers, geriatrics, anemic patients, and
10 patients undergoing corticosteroid (particularly chronic glucocorticoids), chronic NSAID or immunosuppressive therapy.

The particular dosage of a PTHrP analog required to facilitate fracture healing according to this invention will depend on the severity of the condition, the route of administration and related factors which will be decided
15 by the attendant physician. Typically, the dosage will range between about 0.01 and 10 $\mu\text{g/kg}$ body weight per day, preferably from about 0.1 to about 0.5 $\mu\text{g/kg}$ body weight per day. For a 50 kg human subject, the daily dose of active ingredient is from about 0.5 to about 100 μg s, preferably from about 5 to about 10 μg s. This dosage may be delivered in a conventional pharmaceutical
20 composition by a single administration, by multiple applications, or via controlled release, as needed to achieve the most effective results. Dosing will continue for as long as is medically indicated, which depending on the severity of the injury may range from a few weeks to several months.

Unlike most currently available methods of treating fractures, the
25 PTHrP analogs can be administered systemically. Representative delivery regimens include oral, parenteral (including subcutaneous, intramuscular and intravenous), rectal, buccal (including sublingual), transdermal, pulmonary and intranasal. Typical pulmonary and respiratory delivery systems are described in U.S. Patent No. 5,607,915. Nasal delivery of PTHrP analogs is
30 described in WO 97/07815. Also included in the treatment methods of this invention are systemic administration of PTHrP analogs in conjunction with local treatment of a second bone healing agent. Representative agents for such local administration include the bone morphogenetic proteins (BMP-2 and BMP-7), osteogenic proteins (OP-1), growth factors such as TGF- β 1 and
35 cytokines such as IFN- β . Typically these agents are delivered locally in various carriers such as hydroxyapatite and/or calcium carbonate and amylopectin. Systemic administration of PTHrP analogs may also be

combined with alternative methods of fracture healing such as mechanical or biophysical stimulation, e.g., electrical or ultrasound.

PTHrP analogs will typically be administered as pharmaceutical compositions in admixture with a pharmaceutically acceptable, non toxic carrier. As mentioned above, such compositions may be prepared for parenteral (subcutaneous, intramuscular or intravenous) administration, particularly in the form of liquid solutions or suspensions; for oral or buccal administration, particularly in the form of tablets or capsules; for intranasal administration, particularly in the form of powders, nasal drops or aerosols; and for rectal or transdermal administration.

Liquid formulations for parenteral administration may contain as excipients sterile water or saline, alkylene glycols such as propylene glycol, polyalkylene glycols such as polyethylene glycol, oils of vegetable origin, hydrogenated naphthalenes and the like. They may employ slightly acidic buffers in pH ranges of about 4 to about 6. Suitable buffers include acetate, ascorbate and citrate at concentrations ranging from about 5 mM to about 50 mM. For oral administration, the formulation can be enhanced by the addition of bile salts or acylcarnitines. Formulations for nasal administration may be solid and may contain excipients, for example, lactose or dextran, or may be aqueous or oily solutions for use in the form of nasal drops or metered spray. Particular nasal formulations include dry powders suitable for conventional dry powder inhalers (DPI's), liquid solutions or suspensions suitable for nebulization and propellant formulations suitable for use in metered dose inhalers (MDI's). For buccal administration typical excipients include sugars, calcium stearate, magnesium stearate, pregelatinated starch, and the like.

When formulated for nasal administration, the absorption across the nasal mucous membrane may be enhanced by surfactant acids, such as for example, glycocholic acid, cholic acid, taurocholic acid, ethocholic acid, deoxycholic acid, chenodeoxycholic acid, dehydrocholic acid, glycodeoxycholic acid, cyclodextrins and the like in an amount in the range between about 0.2 and 15 weight percent, preferably between about 0.5 and 4 weight percent, most preferably about 2 weight percent.

Delivery of the compounds of the present invention to the subject over prolonged periods of time, for example, for periods of one week to one year, may be accomplished by a single administration of a controlled release system

containing sufficient active ingredient for the desired release period. Various controlled release systems, such as monolithic or reservoir type microcapsules, depot implants, osmotic pumps, vesicles, micelles, liposomes, transdermal patches, iontophoretic devices and alternative injectable dosage forms may be
5 utilized for this purpose. Localization at the site to which delivery of the active ingredient is desired is an additional feature of some controlled release devices, which may prove beneficial in the treatment of certain disorders.

EXAMPLESREFERENCE EXAMPLESTablet formulation

5 The following ingredients are mixed intimately and pressed into single scored tablets.

Quantity per	
Ingredient	tablet, mg
compound of this invention	400
cornstarch	50
10 croscarmellose sodium	25
lactose	120
magnesium stearate	5

Capsule formulation

15 The following ingredients are mixed intimately and loaded into a hard-shell gelatin capsule.

Quantity per	
Ingredient	capsule, mg
compound of this invention	200
20 lactose, spray-dried	148
magnesium stearate	2

Suspension formulation

25 The following ingredients are mixed to form a suspension for oral administration.

Ingredient	Amount
compound of this invention	1.0 g
fumaric acid	0.5 g
sodium chloride	2.0 g

	methyl paraben	0.15 g
	propyl paraben	0.05 g
	granulated sugar	25.5 g
	sorbitol (70% solution)	12.85 g
5	Veegum K (Vanderbilt Co.)	1.0 g
	flavoring	0.035 ml
	colorings	0.5 mg
	distilled water	q.s. to 100 ml

10

Injectable formulation

The following ingredients are mixed to form an injectable formulation.

	Ingredient	Amount
	compound of this invention	0.2 g
	sodium acetate buffer solution, 0.4 M	2.0 ml
15	HCl (1N) or NaOH (1N)	q.s. to suitable pH
	water (distilled, sterile)	q.s. to 20 ml

Nasal formulation

20 The following ingredients are mixed to form a suspension for nasal administration.

	Ingredient	Amount
	PTHrP analog	20 mg/ml
	citric acid	0.2 mg/ml
	sodium citrate	2.6 mg/ml
25	benzalkonium chloride	0.2 mg/ml
	sorbitol	35 mg/ml
	sodium taurocholate or glycocholate	10 mg/ml

EXAMPLE 1

Healing of Segmented Fracture Defects with PTHrP Analogs

A modification of the segmental femoral defect rat model was used to demonstrate that PTHrP analogs facilitate bone healing and fracture repair.

5 (T.A. Einhorn *et al.* J. Bone Joint, 66:274-279 (1984)).

Adult male Sprague-Dawley rats weighing about 300 grams were maintained on Laboratory Rodent Diet 5001 (PMI Feeds, St. Louis, MO, USA) and water *ad libitum*. All rats received only water for 12 hours prior to surgery. The animals were anesthetized prior to surgery by IP injection with
10 ketamine (80 mg/kg) and xylazine (5 mg/kg). A single dose of procaine penicillin was given intramuscularly for prophylaxis against infection.

A lateral approach to the femur was used and a pre-drilled high density polyethylene plate was fixed along the anterior cortex of the femur. During this placement the periosteum of the femur was extensively stripped. A 1 mM
15 non-critical sized segmental defect was created in the mid portion of the femur shaft. The wound was closed with nylon and chromic sutures.

Postoperatively, the rats were placed in cages with 48 hour post-operative access to food pellets. The animals were monitored at least once daily and animals displaying any signs of illness were examined and given
20 appropriate therapy, if necessary.

Rats were divided into groups as shown in Table 1 and either a control vehicle (saline) or a PTHrP analog as described herein was administered subcutaneously daily starting on post-operative day 1. Radiographic analyses were performed weekly. Animals were sacrificed at 6 weeks and compared by
25 radiography.

Sedated rats were placed in the prone position with hindlimbs externally rotated. Serial radiographs of each femur (involved femur and contralateral femur) were taken weekly starting with postoperative week 1. An aluminium phantom device with known density values was included in each radiograph
30 for calibration. Each radiograph was placed on a translumination board and a picture taken using a DCS 420 digital Kodak camera. These images were then transferred to a Gateway 2000 IBM compatible computer and digitized using image analysis software (Sigma Scan®). An outline of the defect was traced and the average density of the area of the osteotomy measured in treated
35 animals relative to control animals. Figure 1 shows the increase in bone

density of the osteotomy in treated animals relative to control animals at weekly intervals over the first five weeks of treatment. The average density of the osteotomy was also compared to the average density of the contralateral femur for each weekly reading. Figure 2 shows the radiographic density ratio after six weeks. Comparison of these bone densities showed that the PTHrP analogs had a bone healing effect. After sacrifice (6 weeks), the femurs (experimental and contralateral) were dissected free of soft tissue. Fine grain radiographs were taken of the femurs in the lateral plane. The osteotomy was considered united when there was osseous continuity of the femur across more than 25% of the cross sectional diameter of the defect. Comparison of femurs from treated animals to control animals showed that PTHrP analogs accelerated bone healing and fracture repair. Figure 3 shows a representative radiograph from control (Fig. 1A) and treated (Fig. 1B) animals.

TABLE 1

Group	Compound	Administration	# of Animals	Sacrifice
1	Control	subcutaneous	10	six weeks
2	PTHrP analog B	subcutaneous	10	six weeks
3	PTHrP analog C	subcutaneous	10	six weeks

EXAMPLE 2

Bone Healing in the Closed Fracture Defect Model with PTHrP Analogs

The closed fracture model used is the Bonnarens/Einhorn model. (F. Bonnarens and T.A. Einhorn, J. Orthopaedic Research, 2:97-101(1984)). The rats used are as in Example 1. In this model an intramedullary rod is placed retrograde through the distal femur via a medial parapatellar approach. The arthrotomy is then closed with non-resorbable sutures and the intramedullary rod is left in place. A closed transverse fracture is then created by a custom made three point clamping device. The animals are monitored and radiographic analysis is performed as in Example 1. PTHrP analogs accelerated bone healing and fracture repair in this model in treated animals relative to control animals.

EXAMPLE 3

Intramembraneous Bone healing with PTHrP analogs

Rabbits were generally anesthetized and subjected to surgery to create four surface defects, one in each distal femur and one in each proximal tibia. A
5 posterior lateral surgical incision measuring approximately 3 cm. was made to expose the distal lateral femoral condyle. Bone proximal to the knee joint was subperiosteally exposed and a 5 mm drill hole was made, keeping the drill bit cool by constant irrigation. The wound was irrigated with normal saline. Deep tissue was closed with a running 3-0 chronic suture followed by closure of the
10 subcuticular layer with a running 3-0 nylon suture and three to four interrupted stainless steel sutures. A second 3 cm. incision was made over the medial proximal tibia and a 5 mm drill hole was made in the proximal medial tibia. The wound was closed as described previously. The entire procedure was repeated on the contralateral limb.

15 Stock solutions of PTHrP analog C, $(\text{MAP}_{1-10})^{22-31}\text{His}^{26}\text{hPTHrP}(1-34) \text{NH}_2$, and PTHrP analog D, $(\text{MAP}_{1-10})^{22-31}\text{hPTHrP}(1-34) \text{NH}_2$, at 800 $\mu\text{g}/\text{ml}$ were prepared by sterile filtration through an 0.22 micron filter and diluted in vehicle to either 20 $\mu\text{g}/\text{ml}$ or 100 $\mu\text{g}/\text{ml}$ just before administration. Vehicle was 30 mg/ml mannitol, 30/mg/ml sucrose, 0.12 mg/ml Tween™ 80, 0.17 mg/ml
20 acetic acid and 2.33 mg/ml sodium acetate trihydrate. PTHrP analogs at either 2 $\mu\text{g}/\text{kg}/\text{day}$ or 10 $\mu\text{g}/\text{kg}/\text{day}$ was administered daily by subcutaneous injection to the surgically treated animals.

Animals were X-rayed weekly for the duration of the study. Densitometric measurements were made using a transilluminating scanner
25 and MetaMorph™ (Universal Imaging, West Chester, PA) software. Radiographs of the lower extremities were performed in internal and external rotation on post-operative day 10 and 21. The animals were sacrificed on post-operative day 30. At the end of the study, animals were euthanized by pentobarbital overdose, tissues were harvested from the left and right femurs
30 and tibias were analyzed by x-ray and histological analysis.

The animals treated with PTHrP analogs C and D showed accelerated intramembraneous bone formation relative to vehicle treated animals. The results for PTHrP analog D are shown in Figures 4A and 4B for the femur and tibia respectively. By day 21 there was no complete healing in either of the
35 femoral or tibial defects in the control treated animals whereas 40% of the of the femoral and tibial defects had healed in the low dose group had healed. At

this same time point, 75% of the tibial defects and 50% of the femoral defects had healed in the high dose group. At sacrifice, radiographic analysis of the specimens confirmed the enhancement of healing in the PTHrP analogue treated animals with over 85% of the high dose tibial defects filled with mineralized tissue while less than 10% of the control tibial defects were filled at sacrifice ($p < 0.01$). Similarly, there was a significant greater percent fill in the PTHrP treated femoral defects than the control treated femoral defects ($p < 0.01$).

10 EXAMPLE 4

Enhanced Bone Healing in the Ulnar Osteotomy Model

The purpose of this experiment was to demonstrate that PTHrP analogs can increase the biomechanical strength (Part I) and the kinetics of healing (Part II) in the context of systemic corticosteroid therapy in a rabbit osteotomy model.

Adult male New Zealand White rabbits were used in all experiments. In Part I, ten rabbits were divided into two equal groups and in Part II, twenty rabbits were divided equally. Non critical sized (1 mm) defects were created bilaterally in each rabbit. From two months prior to surgery through six weeks post-operative, all rabbits received daily subcutaneous injections of either prednisone (0.15 mg/kg) in sterile water (experimental group) or sterile saline (control group). Beginning on the first post-operative day, the experimental group were administered daily sub cutaneous infections of PTHrP analog D (0.01 mg/kg), while the control group received injections of normal saline. In Part I, animals were sacrificed at six weeks after creation of the osteotomy. In Part II, animals were sacrificed once radiographic union was reached bilaterally, or in cases of non-union at ten weeks post-operative.

In both sets of experiments, radiological intensity and healing area were analyzed every other week starting two weeks (Part I) or four weeks (Part II) post-operatively. Serial radiographs of the forelimbs were taken and digitized and the bone area was quantified using image analysis software (Sigma Scan Pro). Photodensitometry was used to quantitate the mineral content of the newly formed bone at sites of osteotomy and callus formation. After sacrifice, high resolution faxitron radiographs were taken of both limbs in the

anteroposterior and lateral planes, allowing for analysis of fracture callus dimension and size.

Results-(Part I)

Nine out ten PTHrP analog D treated limbs achieved union in six weeks, while only two out of ten achieved union in the vehicle treated controls. These results are shown in Figure 5. At six weeks both anteroposterior and lateral faxitrons revealed significantly greater intensities at the osteotomy sites of treated versus control limbs. Similarly, the intensity of both the proximal and distal ulnar diaphyses was significantly greater in the treated limbs. Laterally, the intensity of the external calluses was also greater in the treated limbs. Biomechanically, the torsional strength of the treated limbs was significantly greater than the vehicle-treated control limbs in terms of both stiffness and maximum torque.

Results-(Part II)

At four weeks, the treated limbs demonstrated greater radiographic intensity than the control limbs at the osteotomy site ($p < 0.01$) as well as in the external calluses and ulnar diaphyses. The combined callus areas of the treated limbs was greater ($p < 0.05$) and there was a trend indicating that the osteotomy site was decreasing in size. At six weeks, *in vivo* radiography demonstrated a trend of increased intensity at the osteotomy site of the treated limbs compared to the control limbs, although no differences were observed in callus intensity or area. Radiographically, all the PTHrP analog treated limbs were treated as united and all these animals were sacrificed at six weeks. At eight weeks, four additional limbs (two animals) reached radiographic union and were sacrificed. At ten weeks, the remaining animals were sacrificed, since these limbs showed no radiographic progression towards healing over the preceding four-week period and were thus classified as non-unions. The PTHrP analog treated limbs showed a greater radiographic intensity at the osteotomy site ($p < 0.05$), as well as the ulnar diaphysis and the callus area. The area of the osteotomy was significantly smaller in the PTHrP analog treated limbs than in the vehicle control limbs. Thus in this corticosteroid induced model of delayed healing, PTHrP analog treatment resulted in a complete union rate at six weeks while 75% of untreated limbs showed no tendency to unite at ten weeks.

Claims

1. A method of bone healing and fracture repair comprising administering to a patient in need thereof an effective amount of a polypeptide analog of parathyroid hormone related peptide (PTHrP) and salts thereof, in which
5 amino acid residues 22-31 of said PTHrP analog form an amphipathic α -helix.

2. Use of a polypeptide analog of parathyroid hormone related peptide (PTHrP) and salts thereof, in which amino acid residues 22-31 of said PTHrP analog form an amphipathic α -helix for the preparation of a medicament for bone healing and fracture repair.

10 3. The method of claim 1 or the use of claim 2, wherein the PTHrP analog is administered by nasal delivery.

4. The method of claim 1 or the use of claim 2, further comprising locally administering a second bone healing agent to the fracture.

15 5. The method of claim 1 or the use of claim 2, wherein the fracture is in intramembraneous bone.

6. The method of claim 1 or the use of claim 2, wherein the PTHrP analog is administered systemically.

7. The method of any one of claims 1 or 3-6 or the use of claim 2 or anyone of claims 3-6, wherein the amphipathic α -helix is

20 Glu Leu Leu Glu Xaa Leu Leu Glu Lys Leu

wherein Xaa is a positively charged amino acid residue.

8. The method or use of claim 7, wherein Xaa is lysine or histidine.

9. The method or use of claim 8, wherein the PTHrP analog is:

25 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu
Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂
(SEQ ID NO:3) or

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu
Arg Arg Arg Glu Leu Leu Glu His Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ
ID NO:4).

30 10. The method or use of claim 7, wherein the PTHrP analog has at least two amino acid residues linked via their side chains to each other.

11. The method or use of claim 10, wherein the linked amino acid residues are at positions 13 and 17 or at positions 26 and 30.

12. The method or use of Claim 11, wherein the amino acid residue at position 17 is an aspartic acid.

5 13. The method or use of Claim 12, wherein the PTHrP analogs are:

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu
Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr hSerlac (SEQ
ID NO:5) or

10

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu
Arg Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂
(SEQ ID NO:6).

14. The method of any one of claims 1 or 3-6 or the use of claims 2 or
15 anyone of claims 3-6, wherein the PTHrP analog is an N-terminal truncate of
30 to 50 amino acid residues with optionally substituted at positions 5, 13, 17,
19, 26, 30, 32 and/or 34.

15. The method or use of Claim 14, wherein the PTHrP analog is:

Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
20 Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
NO:7),

Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Ala
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr Ala NH₂ (SEQ ID
NO:8),

25 Ala Val Ser Glu Ile Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Ala
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu Pro NH₂ (SEQ ID NO:9),

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr D-Orn
lactam (SEQ ID NO:10),

30 Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr hSer lactam (SEQ
ID NO:11) or

Ala Val Ser Glu His Gln Leu Leu His Asp Lys Gly Lys Ser Ile Gln Asp Leu Arg
Arg Arg Glu Leu Leu Glu Lys Leu Leu Glu Lys Leu His Thr hSer Thr His Ile
Gln NH₂ (SEQ ID NO 12).

1/6

Figure 1

PTHrP Study - Defect

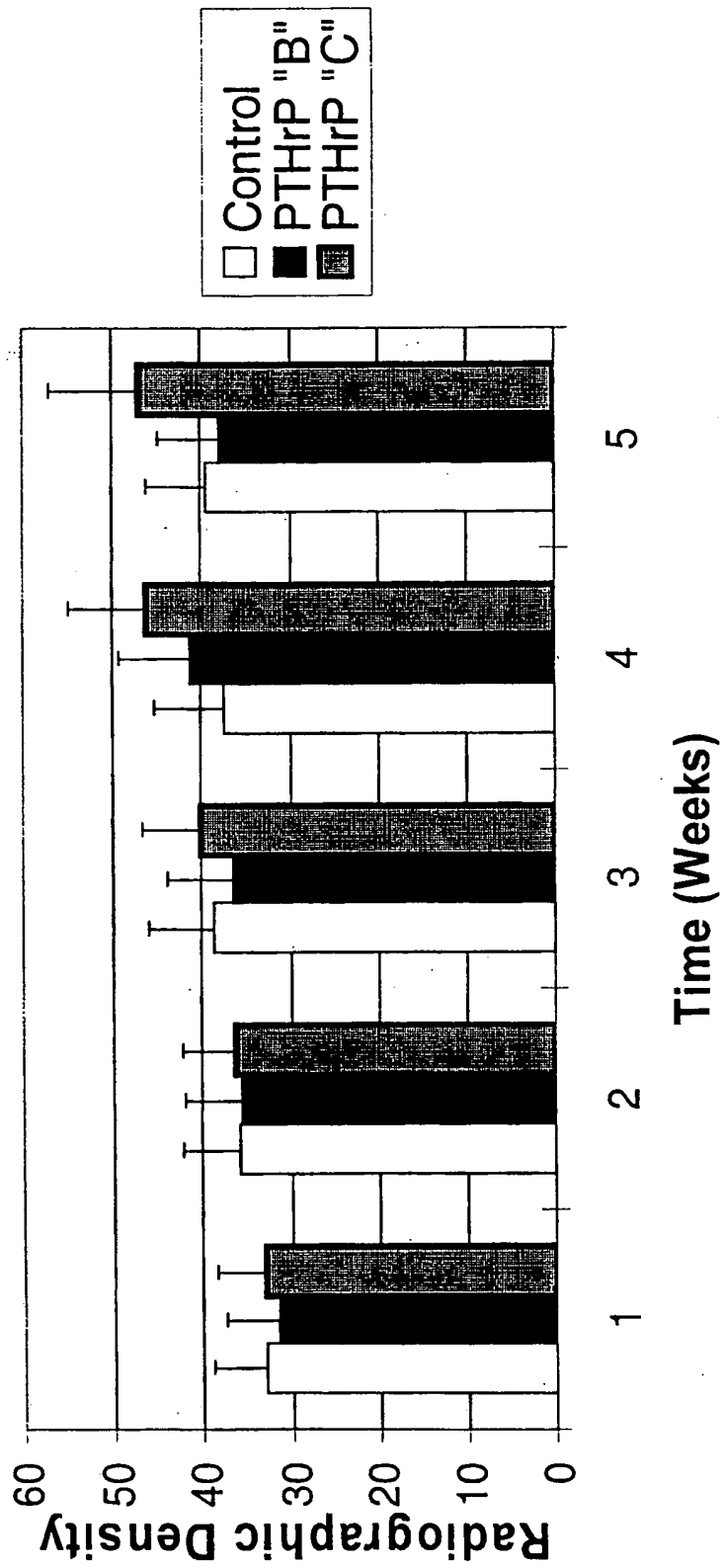


Figure 3A Example of a high resolution radiograph of non-united defect at 6 weeks

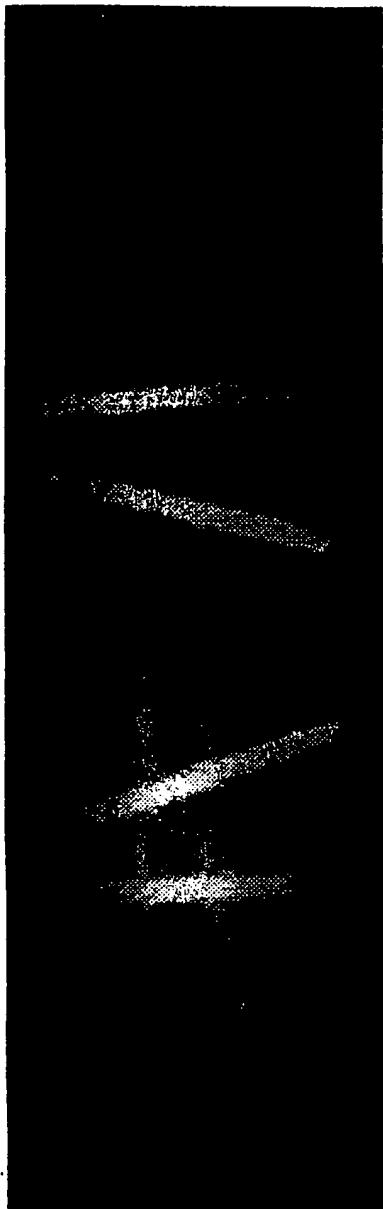


Figure 3B Example of a high resolution radiograph of a healed defect at 6 weeks

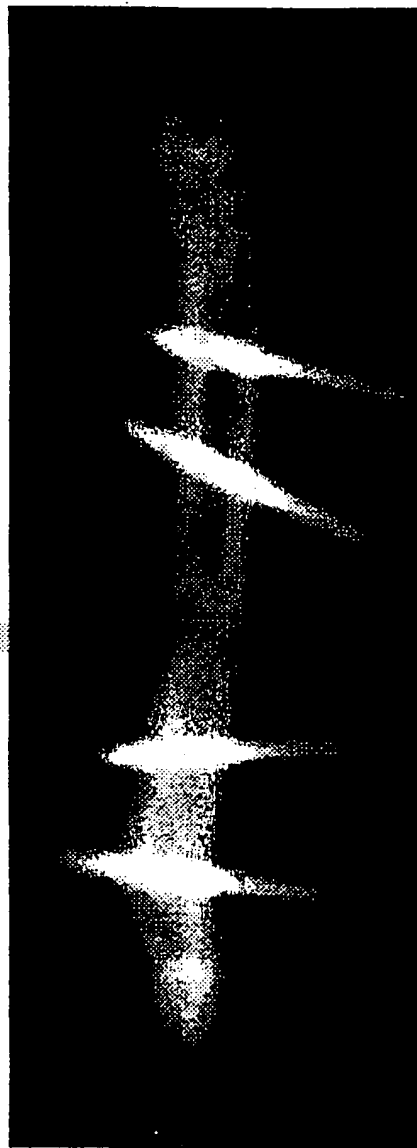


Figure 4A

Trabecular Bone of Femur

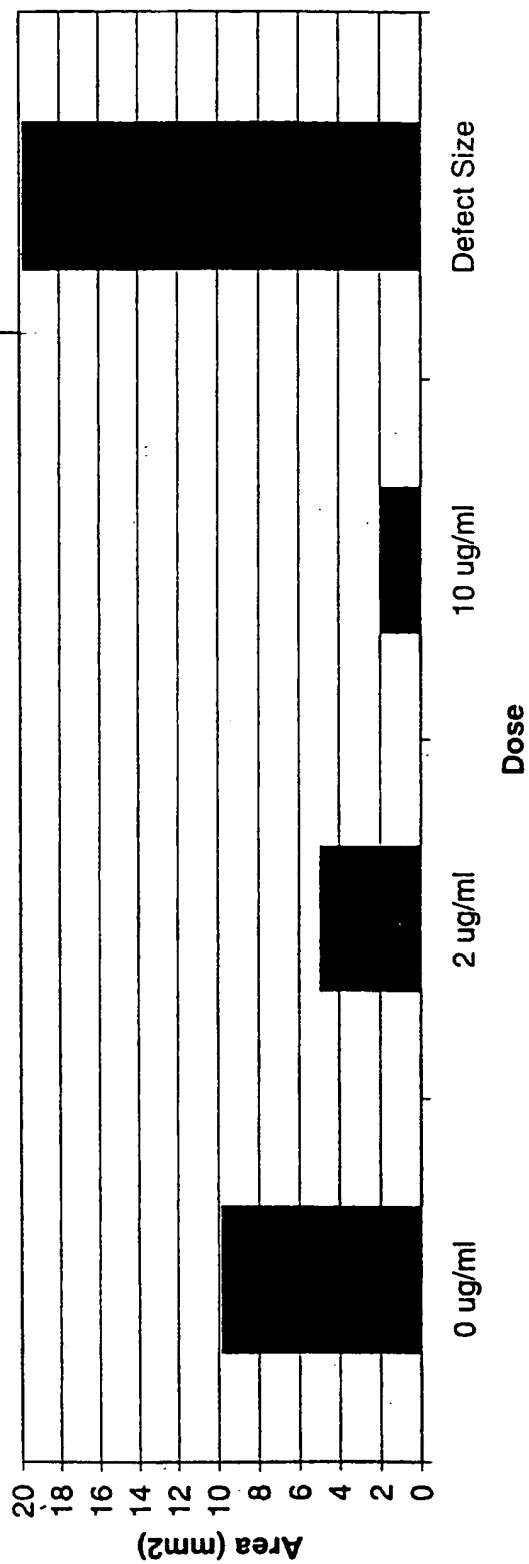
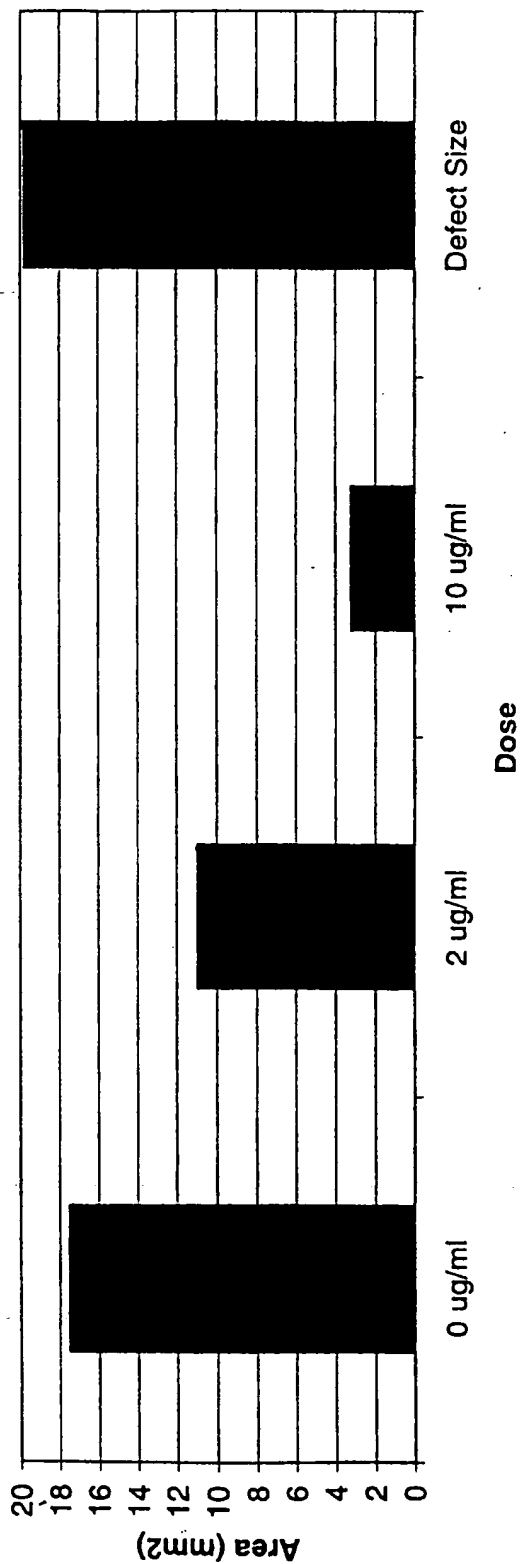


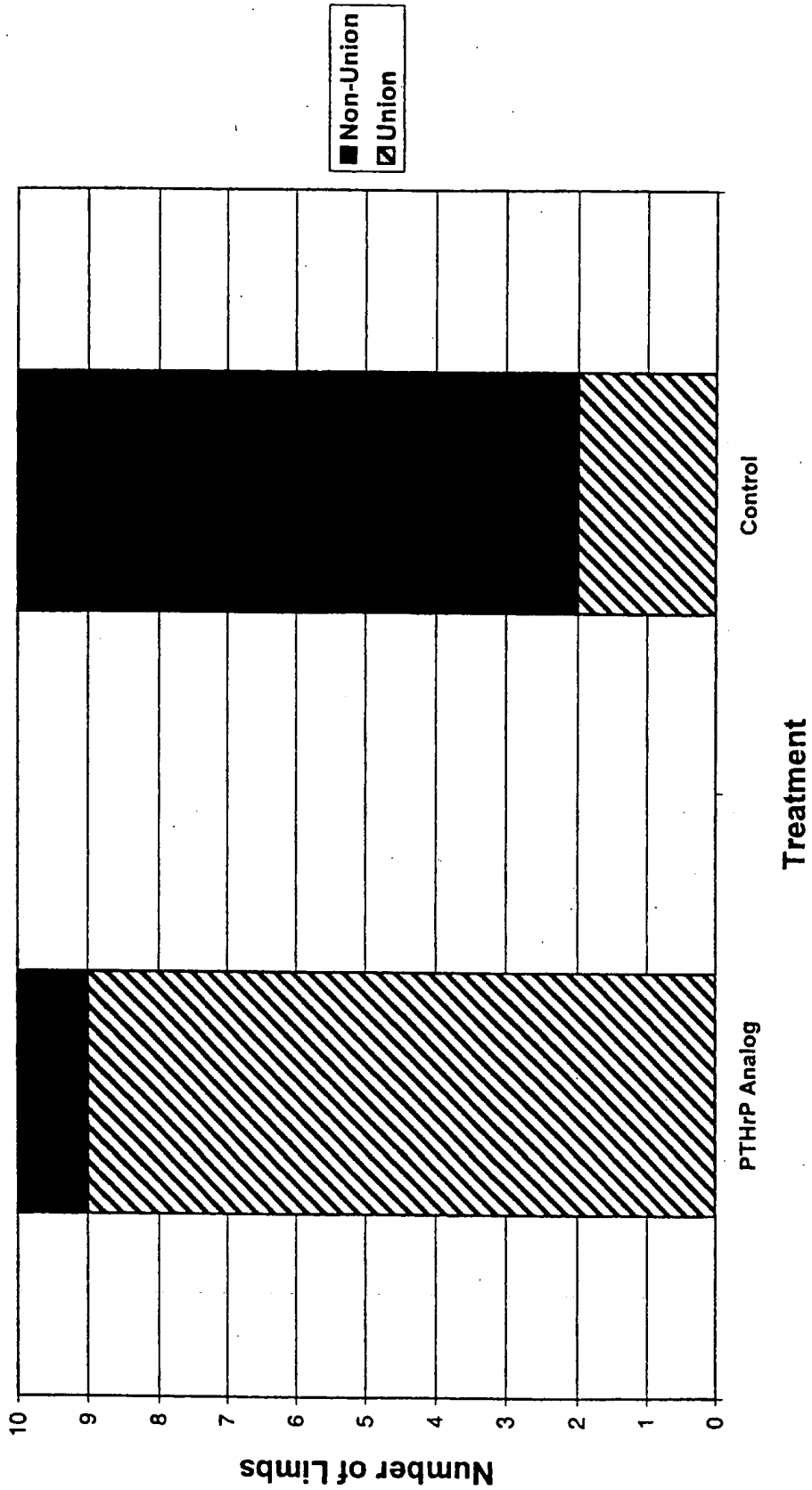
Figure 4B

Cortical Bone of Tibia



Union Rate at 6 Weeks

Figure 5



**This Page is Inserted by IFW Indexing and Scanning
Operations and is not part of the Official Record**

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- ☐ BLACK BORDERS
- ☐ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
- ☒ FADED TEXT OR DRAWING
- ☐ BLURRED OR ILLEGIBLE TEXT OR DRAWING
- ☐ SKEWED/SLANTED IMAGES
- ☐ COLOR OR BLACK AND WHITE PHOTOGRAPHS
- ☐ GRAY SCALE DOCUMENTS
- ☒ LINES OR MARKS ON ORIGINAL DOCUMENT
- ☐ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY
- ☐ OTHER: _____

IMAGES ARE BEST AVAILABLE COPY.

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.